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Research paper

Differences of C sequestration in functional groups of soil humic acid under long term application of manure and chemical fertilizers in North China

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ABSTRACT

The differences of molecular characteristic of humic acid (HA) under long-term application of chemical fertilizer and manure for 34 years were determined by ^{13}C cross polarization magic angle spinning nuclear magnetic resonance (^{13}C CPMAS NMR) spectroscopy in order to investigate the carbon (C) sequestration in soils. The results showed that the relative content of alkyl C increased in manure treatments, compared to control and chemical fertilizer treatments. There were positive correlations between alkyl C and hydrogen to carbon (H/C) ratio ($R^2 = 0.84$, $P < 0.01$), alkyl C and amounts of HA ($R^2 = 0.57$, $P < 0.01$) as well as alkyl C and E4/E6 ratio ($R^2 = 0.55$, $P < 0.01$). Conversely, negative correlations were found between aromatic C and alkyl C ($R^2 = 0.76$, $P = 0.01$), H/C ratio and aromaticity ($R^2 = 0.37$, $P = 0.04$), aromaticity and amounts of HA ($R^2 = 0.71$, $P < 0.01$) as well as aromaticity and E4/E6 ratio ($R^2 = 0.65$, $P < 0.01$). Compared to control treatment, the amorphous C/crystalline C ratio increased for 0.02–0.14 and 0.30–0.78 in chemical fertilizer treatments and manure treatments, separately. Moreover, the short-chain $(CH_2)_n$/Long-chain $(CH_2)_n$ ratio increased for 0.03–0.37 and 0.03–0.18 in chemical fertilizer treatments and manure treatments, separately.

These findings suggest that application of manure favours alkyl C sequestration in HA and tends to be more closely linked to amorphous C than crystalline C, while incorporates less short-chain $(CH_2)_n$, compared to chemical fertilizer treatments. Moreover, the E4/E6 ratio of HA increased with more alkyl C.

1. Introduction

Carbon (C) sequestration in soils offers opportunity to mitigate greenhouse gases emissions from agriculture. The knowledge of stabilization and decomposition processes of organic matter in different soils is necessary in order to understand, assess and predict effects of land use changes on the storage and stability of soil carbon (Paul et al., 2008). It has been suggested that organic C stabilization is dominated by the selective preservation of recalcitrant organic components that accumulate in proportion to their chemical properties (von Lützow et al., 2006). Soil organic carbon (SOC) can be divided into humic acid (HA), fulvic acid (FA) and humin (HU) by chemical fractionation (von Lützow et al., 2007). There are many studies on the molecular structure characteristics of HA analyzed by non-destructive spectroscopic method such as ^{13}C cross polarization magic angle spinning nuclear magnetic resonance (^{13}C CPMAS NMR) spectra (Nardi et al., 2007; Spaccini and Piccolo, 2007; Bartoszek et al., 2008). Moreover, the selective preservation of hydrophobic alkyl molecules of HA like long chain fatty acid is also studied (Spaccini and Piccolo, 2009). The hydrophobic components of organic matter may have an essential role in controlling the processes of organic carbon sequestration in paddy soils of South China (Song et al., 2013).

The SOC increased by long term use of manure or chemical fertilizer in the eastern of China (Wang et al., 2015). Organic fertilizers (manures and slurries) applied repeatedly over many cropping seasons favourably influence nutrient recycling, maintenance of soil organic matter (SOM), and improve soil quality parameters such as soil aggregation and porosity (Domingo-Olivé et al., 2016). However, the hydrophobic fractions and hydrophilic fractions of HA under long-term fertilization of manure can promote C sequestration, especially involving different forms of

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alkyl C. The crystalline C is more stable than amorphous C (Hu et al., 1999), even though both of them are alkyl C. The HA structures tend to be more simple and high aliphaticity under application of organic matter (Dou et al., 2008). But the form of increased aliphatic C is not clear. Not only alkyl C, but also aromatic C of HA is hydrophobic components. The N addition will increase C sequestration by slowing the decomposition of SOM, as well as stabilizing SOM against microbial decomposition in aggregate-occluded pools (Riggs et al., 2015). Moreover, the high content of aromatic C and phenolic C of humus could increase by long term N application (Sjöberg et al., 2004). However, the differences of C sequestration in hydrophobic components of HA, including alkyl C and aromatic C, under long term application of manure and chemical fertilizer are not clear.

The objective of this work was therefore to apply ^{13}C CPMAS NMR spectroscopy to evaluate the molecular changes of HA under long term fertilization with manure and chemical fertilizer, with a special focus on the differences of hydrophobic components of HA.

2. Materials and methods

2.1. Site and sampling

The experimental site was located on Eutric Cambisols (FAO/UNESCO, 1997) without calcium carbonate in the Laiyang region of China (36.9°N, 120.7°E). It is a warm temperate zone with a mean annual temperature of 11.2 °C and mean annual rainfall of 779.1 mm. The site was established for fertility assessments and the evaluation of fertilizer efficiency and included nine treatments which were started in 1978. Three replicate 33 m^2 plots were assigned to each treatment. The treatments were: unfertilized (control), low rate of N fertilizer (N1), high rate of N fertilizer (N2), low rate of organic manure (M1), low rate of organic manure and nitrogen (N) fertilizer (M1N1), low rate of organic manure with high rate of N fertilizer (M1N2), high rate of organic manure (M2), high rate of organic manure with low rate of N fertilizer (M2N1), high rate of organic manure and N fertilizer (M2N2), In addition, three treatments were added in 1984. The addition treatments were: high nitrogen fertilizer with phosphorus and potassium fertilizer (N2PK), high nitrogen fertilizer with phosphorus (N2P) and high nitrogen fertilizer with potassium (N2K) (Table 1). A long crop rotation of wheat (sowed in October) and maize (sowed in June) was evaluated. The wheat and maize straw were removed after harvest. In addition, the root of maize was removed, but the root of wheat was left in the field. Soil samples were mixed by five samples in one plot, and then saved about 1 kg samples for further analysis after wheat harvest in June 2011. In this study, a sample was mixed by three replicates. The properties of soil before field experiments were shown in Table 2.

Table 1
Fertilization rates in the experiment field per year (kg ha^{-1}).

Treatments	Pig manure	Inorganic fertilizer		
		N	P_2O_5	K_2O
Control	0	0	0	0
N1	0	138	0	0
N2	0	276	0	0
N2PK	0	276	90	135
N2P	0	276	90	0
N2K	0	276	0	135
M1	30,000	0	0	0
M1N1	30,000	138	0	0
M1N2	30,000	276	0	0
M2	60,000	0	0	0
M2N1	60,000	138	0	0
M2N2	60,000	276	0	0

2.2. Extraction of humic substances

The HA were extracted and divided using methods described by Zhang et al. (2011) and Dou et al. (2008) with minor revision. The following fractions were obtained: The soil was extracted by distilled water (1:10, w/v) which was water dissolved substances (WSS). Then the humic extractable substances (HE) were extracted for 24 h by 0.1 M NaOH and 0.1 M $Na_4P_2O_7$ solution (50:50, v/v). The HE was brought to pH 1 with 0.5 M H_2SO_4 and the HA was allowed to precipitate at 20 °C, while the solution contained FA. The soil residue was humin and was washed by distilled water to bring it to pH 7, and then air-dry and sieved at 0.25 mm.

2.3. Purification of humic substances

The HA were transferred into the centrifuge bottle and centrifuged at approximately 7150*g* for 20 min at 20 °C and filtered (0.22 μm) into a volumetric flask, then it was dialyzed against deionized water until Cl^- free, and freeze-dried.

2.4. Chemical and physical analysis

The amounts of SOC were determined with dichromate using the wet oxidation method (Nelson and Sommers, 1982). In addition, pH and the content of total nitrogen (N), Olsen phosphorus (P), exchangeable potassium (K), cation exchange capacity (CEC) and bulk density was analyzed by standard methods and described in detail by Lu (2000). Basically, soil pH was measured with a composite electrode (Mettler Toledo Seveneasy Precision pH meter, Switzerland, 2006) in a 1:2.5 soil-water ratio. Total Kjeldahl N was determined after Kjeldahl digestion and titration using a modified protocol by Lu (2000). Exchangeable K was extracted with 1 M ammonium acetate (pH 7) and measured by flame photometer. Cation exchange capacity was measured with leaching with 1 M ammonium acetate (pH 7). Olsen-P was extracted with 0.5 M sodium bicarbonate and measured by spectrophotometer. Moreover, soil bulk density and total P were determined by traditional methods.

2.5. Solid-state NMR spectroscopy

The ^{13}C CPMAS spectra were recorded on a Bruker AVANCE III 400 WB spectrometer equipped with a 4 mm standard bore CPMAS probehead whose X channel was tuned to 100.62 MHz for ^{13}C and the other channel was tuned to 400.18 MHz for broad band 1H decoupling, using a magnetic field of 9.39 T at 297 K. The dried and finely powdered samples were packed in the ZrO_2 rotor closed with Kel-F cap which were spun at 5 kHz rate. The experiments were conducted at a contact time of 2 ms. A total of 10000 scans were recorded with 6 s recycle delay for each sample. All ^{13}C CPMAS chemical shifts are referenced to the resonances of adamantane ($C_{10}H_{16}$) standard ($dCH_2 = 38.5$).

The overall chemical shift range of ^{13}C CPMAS NMR spectra of HA was divided into the following main resonance regions: alkyl C (0–45 ppm); N—C (45–60 ppm); oxygen alkyl C (O-alkyl-C) (60–110 ppm); aromatic-C (110–160 ppm) including aryl C (110–145 ppm) and phenol C (145–160 ppm); carboxyl- and carbonyl-C (160–190 ppm).

3. Results

3.1. Elemental changes of HA

In this study, elemental composition of HA were shown in Table 3. Compared to control treatment, the H/C ratio increased for 0.04–0.06 and 0.07–0.11 in manure and chemical fertilizer treatments, separately. The C/N ratio decreased for 0.14–0.83 in manure or chemical fertilizer treatments, except N2PK and N2P treatments. In addition, C/N ratio